PhytoKeys

PhytoKeys 243: 231–248 (2024)
DOI: 10.3897/phytokeys.243.114415

Research Article

Two new species of *Paraphlomis* (Lamiales, Lamiaceae) from limestone karsts in Guangdong Province, China

Wan-Yi Zhao[1], Qin-Dai Xiong[1], Rang-Min Wu[2], You-Hong Zeng[3], Zhi-Bin Xie[4], Ya-Ping Chen[5], Qiang Fan[1]

1 *State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Stress Biology, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China*
2 *Qingyuan Forestry Bureau, Qingyuan 511518, China*
3 *Qingyuan Jinji Forestry Farm, Qingyuan 513029, China*
4 *Yingde Forestry Bureau, Qingyuan 513099, China*
5 *CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China*
Corresponding author: Qiang Fan (fanqiang@mail.sysu.edu.cn)

Abstract

Paraphlomis qingyuanensis and *P. baiwanensis* (Lamiaceae), two new species from the limestone area in Guangdong Province, China, are described. Morphologically, both species belong to *P.* ser. Subcoriaceae C.Y. Wu & H.W. Li. A close relationship between the two new and *P. subcoriacea* was revealed by molecular phylogenetic analyses based on ETS and ITS. Further morphological and population genetic evidence indicated that they are distinct species in *Paraphlomis*. According to the IUCN Red List Categories and Criteria, *P. qingyuanensis* and *P. baiwanensis* were assessed as Endangered (EN) and Deficient (DD), respectively.

Key words: IUCN, limestone, new taxon, Paraphlomideae, phylogeny, Qingyuan, RAD-seq

OPEN ACCESS

Academic editor: Alan Paton
Received: 19 October 2023
Accepted: 13 June 2024
Published: 27 June 2024

Citation: Zhao W-Y, Xiong Q-D, Wu R-M, Zeng Y-H, Xie Z-B, Chen Y-P, Fan Q (2024) Two new species of *Paraphlomis* (Lamiales, Lamiaceae) from limestone karsts in Guangdong Province, China. PhytoKeys 243: 231–248. https://doi.org/10.3897/phytokeys.243.114415

Copyright: © Wan-Yi Zhao et al.
This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

Introduction

Paraphlomis (Prain) Prain is a member of the tribe Paraphlomideae Bendiksby (Lamiaceae, Lamioideae) (Bendiksby et al. 2011; Li et al. 2016; Zhao et al. 2021). Most species of *Paraphlomis* are distributed in southern China, with several species occurring in the Himalayas, Korea and Southeast Asia (Li and Hedge 1994; Wu and Li 1997; Ko et al. 2014; Chen et al. 2021). Previous molecular phylogenetic studies of *Paraphlomis* revealed that the genus was not monophyletic, because species of *Matsumurella* were recovered within it (Chen et al. 2021; Chen et al. 2022b; Guo et al. 2023). In general, *Paraphlomis* is characterized by its herbaceous habit, actinomorphic calyx with five lobes less than half as long as the tube, corolla 2-lipped (1/3) with hairy upper lip but hardly bearded along the margin, included stamens and an apically truncate ovary (Wu and Li 1977; Bendiksby et al. 2011; Ko et al. 2014; Chen et al. 2021).

As currently circumscribed, a total of 37 species and seven varieties are recognised within *Paraphlomis* (Li and Hedge 1994; Chen et al. 2022a, 2022b;

Yuan et al. 2022; Guo et al. 2023; Yan et al. 2023). China is the distribution center of *Paraphlomis*, with 23 species documented in the Flora of China (Li and Hedge 1994). In recent years, a number of new species and infraspecies of *Paraphlomis* were reported in China, including *P. javanica* var. *pteropoda* D. Fang & K.J. Yan and *P. javanica* var. *angustifolia* f. *albinervia* D. Fang & K.J. Yan (Yan and Fang 2009); *P. breviflora* B.Y. Ding, Y.L. Xu & Z.H. Chen (Ding et al. 2019); *P. kuankuoshuiensis* R.B. Zhang, D. Tan & C.B. Ma (Zhang et al. 2020); *P. jiangyongensis* X.L. Yu & A. Liu and *P. coronata* (Vaniot) Y.P. Chen & C.L. Xiang (Chen et al. 2021); *P. nana* Y.P. Chen, C. Xiong & C.L. Xiang (Chen et al. 2022c); *P. longicalyx* Y.P. Chen & C.L. Xiang (Chen et al. 2022a); *P. hsiwenii* Y.P.Chen & XiongLi (Chen et al. 2022b); *P. strictiflora* J.C.Yuan, B.Chen & C.L.Xiang (Yuan et al. 2022); *P. jinggangshanensis* Boufford, W.B. Liao & W.Y. Zhao (Zhao et al. 2022); *P. yingdeensis* W.Y.Zhao, Y.Q.Li & Q.Fan (Guo et al. 2023), *P. caloneura* K.J.Yan, Y.P.Chen & Y.Feng Huang (Yan et al. 2023).

During a botanical expedition of the limestone area in Qingyuan city, Guangdong Province in 2023, we discovered two unknown species of *Paraphlomis*. The calyx teeth of the two unknown species extended into wings from veins, which are consistent with the characteristics of *Paraphlomis* ser. Subcoriaceae C.Y. Wu et H.W. Li (Li 1965; Wu and Li 1977). However, their morphological characters differ from the two known two species of the series, *P. subcoriacea* C. Y. Wu ex H. W. Li and *P. brevifolia* C. Y. Wu & H. W. Li. Thus, we suspected both of them were undescribed species. After careful field observations, morphological comparisons with other species of *Paraphlomis*, and molecular phylogenetic studies, we confirmed that they were new species and named them as *P. qingyuanensis* W.Y. Zhao, R.M. Wu & Q. Fan and *P. baiwanensis* W.Y. Zhao, Y.P. Chen & Q. Fan.

Materials and methods

Morphological study

The flowering and fruiting plants of the two new species were examined in the field from August to December in 2023 and compared with herbarium specimens deposited in IBSC, KUN, GCMI and SYS (herbarium acronyms follow Thiers 2023). The two putative new species were most similar to *Paraphlomis subcoriacea* and *P. brevifolia*. We have carried out several field work trips to the collection site of the type specimens of these two species from December 2023 to April 2024. Unfortunately, we did not find *P. brevifolia* in the field due to a lack of detailed collection site information (Li 1965). Therefore, the morphological features contrasting with those of *P. brevifolia* were based on its type specimens (IBSC0005124). All morphological characteristics were measured using dissecting microscopes.

Phylogenetic analyses

The nuclear ribosomal internal and external transcribed spacers (ITS and ETS) were used for reconstructing the phylogeny of the suspected new species and related taxa based on previous study (Chen et al. 2021; Zhao et al. 2022).

Most sequences were downloaded from GenBank, except for the two nuclear ribosomal DNA (nrDNA) sequences of the two new species and *Paraphlomis subcoriacea*, which were newly sequenced in the present study. Genomic DNA of the suspected new species was extracted from silica-gel-dried leaves using the modified 2× CTAB procedure of Doyle and Doyle (1987). The ITS and ETS sequences were amplified with primer pairs 17SE/26SE (Sun et al. 1994) and ETSB/IGS (Beardsley and Olmstead 2002), respectively, with PCR amplification and sequencing following Chen et al. (2016). A total of 49 accessions representing 30 species and four varieties/subspecies of *Paraphlomis* and two species of *Matsumurella* were sampled in the phylogenetic study. *Phlomoides bracteosa* (Royle ex Benth.) Kamelin & Makhm. and *Phlomis fruticosa* Sieber ex C. Presl were selected as the outgroups. The GenBank accession numbers are listed in Appendix 1.

Nucleotide sequences were aligned using MAFFT 7 (Katoh and Standley 2013). After removing aligned columns with more than 70% missing data using Phyx (Brown et al. 2017), the two nrDNA regions were concatenated for phylogenetic reconstruction. The phylogenetic relationships were assessed using the Bayesian inference (BI) and maximum likelihood (ML) methods, and both were implemented on the online server Cyberinfrastructure for Phylogenetic Research Science (CIPRES) Gateway (http://www.phylo.org/; Miller et al. 2010). The BI analysis was carried out using MrBayes 3.2.7a (Ronquist et al. 2012) and the ML analysis using RAxML-HPC2 (Stamatakis 2014), with detailed settings following that of Chen et al. (2021). The resulting BI tree with posterior probabilities (PP) and best-scoring ML tree with bootstrap support (BS) values were visualized and annotated using TreeGraph 2 (Stover and Müller 2010).

Population genetic structure analyses

To investigate the phylogenetic relationship between the two newly discovered species and their close relatives, we further conducted an analysis of their population genetic structure. A total of 84 individuals were sampled from seven populations of the two putative *Paraphlomis* new species and its close relative *P. subcoriacea* (Appendix 2). The fresh leaves were dried and stored with silica gel, and then sent to JieRui BioScience Co. Ltd. (Guangzhou, China) for DNA extraction, ddRAD-seq library preparation, and Illumina sequencing. The produced raw data was processed with the software Stacks 2.55 (Catchen et al. 2013). The procedure “process_radtags” was used to demultiplex RAD tags, “denovo_map.pl” to process all the fastq files, and “populations” to filter the data by setting “--min-maf 0.05 --max-obs-het 0.7 -R 0.8 –write-random-snp –vcf”. The produced vcf file was transformed to ped file using bed file using software vcftools 0.1.16 (Danecek et al. 2011), and to bed file using the software Plink v1.90 (Chang et al. 2015). The produced bed file was used to perform Bayesian cluster analysis with the software ADMIXTURE v1.3.0 (Alexander et al. 2009), in which the number of groups (K) was set from 1–6 and the optimal K was determined by the minimum value of cross-validation error (CV). Principal coordinate analysis (PCA) was performed with Plink, and python script were used to draw the scatter diagram.

Results and discussion

The combined nuclear data set was 1211 bp (773 bp for ITS, 438 bp for ETS) in length, including 347 variable sites (165 for ITS, 182 for ETS) and 200 parsimony-informative characters (87 for ITS, 113 for ETS). The resulting phylogenetic tree of *Paraphlomis* in this study was similar to that in previous studies (Chen et al. 2021; Zhao et al. 2022). The two accessions of *P. baiwanensis* grouped together (Fig. 1: BS = 100%/PP < 0.50) and this new species was sister to *P. subcoriacea* (Fig. 1: BS = 100%/PP = 1.00). The *P. baiwanensis*-*P. subcoriacea* clade was further sister to another new species, *P. qingyuanensis* (Fig. 1: BS = 99%/PP = 1.00). All three species were nested within the previously suggested "Clade III" by Chen et al. (2021) (Fig. 1: BS = 78%/PP = 1.00). Species of "Clade III" (marked in blue in Fig. 1) are characterized by hairy nutlets/ovaries (Chen et al. 2021; 2022b). The nutlets of both new species are sparsely hispid and densely glandular at apex (Figs 2M, 5I, 6H), which confirmed the significance of nutlet morphology for the infrageneric classification of *Paraphlomis* (Chen et al. 2021).

The close relationships among the two new species and *P. subcoriacea* are also supported by morphological evidence. In morphology, the two putative new species are most similar to *Paraphlomis brevifolia* C. Y. Wu et H. W. Li and *P. subcoriacea* C. Y. Wu ex H. W. Li. They share the features such as conspicuously extended calyx teeth, and the fact that they are also growing in a limestone habitat. A comparison of their morphological features is presented in Table 1 and Fig. 4.

The putative new species *Paraphlomis qingyuanensis* differs from *P. brevifolia* by its slender and much branched stem (vs. unbranched) (Figs 2C, 4A), leaves without glandular (vs. abaxially golden glandular) (Table 1). Furthermore, the reticulate veins and pilose indumentum on leaves of *P. brevifolia* are more obvious (Fig. 4B). *P. qingyuanensis* could also be easily distinguished from *P. subcoriacea* by its papery leaves (vs. thin leathery), fewer leaf veins, 2–4 pairs (vs. 5–6 pairs), and smaller leaf size (Table 1, Fig. 4A, D).

Paraphlomis baiwanensis is most similar to *P. subcoriacea*. They share such features as leaves lanceolate, 5–7 lateral vein pairs, and leaf base shape (Table 1). However, *P. baiwanensis* could be easily distinguished from the latter by its stout stem (diam. 3.6–5.3 mm vs. ca. 3.6 mm), leathery leaves (vs. thin leathery), and larger leaves size (12.5–18.7 cm × 2.6–4.8 cm vs. 7–15 cm × 1.5–3.2 cm) (Table 1, Fig. 4C, D). Furthermore, the distribution areas of the two species are separated by a distance of approximately 45 km, exhibiting complete non-overlap (Fig. 7A). The results of population genetic analysis also confirmed significant differences in their genetic structure (Fig. 7B).

Bayesian cluster analysis showed lowest CV value as $K = 3$, each of the three species *P. baiwanensis* (P1), *P. subcoriacea* (P2–4), and *P. qingyuanensis* (P5–7) possesses a unique gene pool, and no gene admixture is observed in any individuals (Fig. 7B). PCA analysis reveals similar results in which the 84 individuals are assigned into three groups and individuals of the same species are clustered into the same group (Fig. 7C). These results showed that individuals of the three species can be separated clearly from each other based on genomic data, strongly supported their species status and no obvious gene flow was observed among the three species.

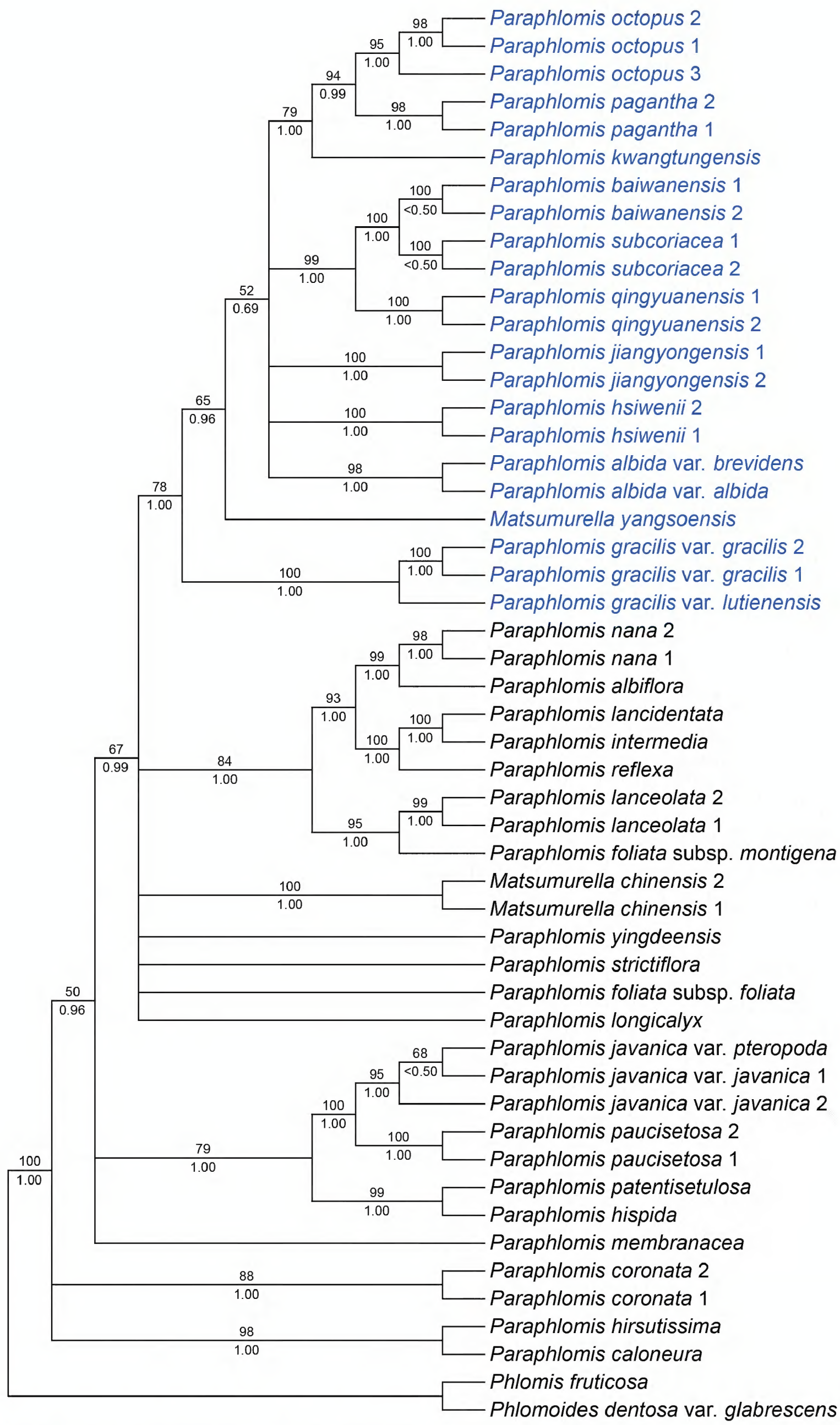

Figure 1. The best-scoring maximum likelihood tree of *Paraphlomis* inferred from concatenated nrDNA (ETS and ITS) dataset. Support values ≥ 50% BS or 0.50 PP are displayed above and below the branches, respectively. Multiple accessions of the same species are numbered according to Appendix 1.

Table 1. Morphological comparisons among *Paraphlomis baiwanensis*, *P. brevifolia*, *P.qingyuanensis*, and *P. subcoriacea*.

Characters	*P. qingyuanensis*	*P. baiwanensis*	*P. subcoriacea*	*P. brevifolia*
Stem	50–90 cm, slender, 1.4–3.1 mm in diam	50–80 cm tall, erect, stout, 3.6–5.3 mm in diam	ca. 60 cm, ca. 3.6 mm in diam	ca. 40 cm, ca. 2.5 mm in diam
Stem branching	much branched	unbranched or 2–3 branched	unbranched or 2–3 branched	unbranched
Length of petiole	0.4–1.6 cm	0.6–1.3 cm	1.0–1.3 cm	0.3–0.6 cm
Leaf size	2.7–9.5 cm long, 1.2–2.5 cm wide	12.5–18.7 cm long, 2.6–4.8 cm wide	7–15 cm long, 1.5–3.2 cm wide	5–8 cm long, 2.4–3.4 cm wide
Leaf base	cuneate, not decurrent	cuneate or abruptly obtuse, not decurrent	attenuate or abruptly obtuse, not decurrent	obtuse to rounded, not decurrent
Leaf texture	papery	leathery	thin leathery	thin leathery
Leaf vein	2–4 (-5) pairs	5–7 (-8) pairs	5–6 pairs	4–5 pairs
Calyx teeth	subtruncate, extended into wings from veins	subtruncate, extended into wings from veins	subtruncate, extended into wings from veins	triangular, conspicuously extended into wings from veins
Corolla	upper lip yellow, lower lip red with purple spots	white	white or purple-white	unknown

Taxonomic treatment

***Paraphlomis qingyuanensis* W.Y.Zhao, R.M.Wu & Q.Fan, sp. nov.**
urn:lsid:ipni.org:names:77344362-1
Figs 2, 3, 4A
清远假糙苏

Type. China. Guangdong Province: Yingde City, Huanghua Town, near Hegushi, on the limestone valley, 24°13'N, 112°56'E, alt. 135 m, 7 September 2023, *Xiong Qin-Dai ZWY-3793* (holotype: SYS00236954! isotypes: KUN, SYS00236955!, SYS00236968!, SYS00236969!).

Diagnosis. *Paraphlomis qingyuanensis* is morphologically similar to *P. brevifolia* and *P. subcoriacea*, but differs from the latter two species by its slender and much branched stem, papery leaves, inconspicuous reticulate veining, and fewer leaf veins.

Description. ***Herbs*** perennial, 50–90 cm tall; stoloniferous, with sparsely villous. **Stems** erect or decumbent, slender and much branched, 4-angled, diameter 1.4–3.1 mm, densely retrorse pilose. ***Leaves*** opposite; lamina ovate to oblong, papery, 2.7–9.5 cm long, 1.2–2.5 cm wide, apex obtuse, base cuneate, margin crenate-serrate; adaxially green, abaxially light green, sparsely pilose on both sides, more densely on viens; lateral veins 2–4 (-5)-paired; petiole 0.4–1.6 cm long, densely pilose. ***Verticillasters*** born in upper leaf axils, cymes 1–4-flowered; bracteoles inconspicuous, ca. 0.5 mm long, early deciduous; pedicels ca. 1 mm long, sparsely pilose. ***Calyx*** light green, obconical, 4.5–5 mm long, outside with sparsely pilose and white glandular, glabrous inside, conspicuously 10-veined; teeth 5, inconspicuous so that calyx mouth appears subtruncate, extended into wings from veins as 1.5–2.0 mm long. ***Corolla*** 1.0–1.2 cm long; tube ca. 0.3 cm long, ca. 1 mm in diam., straight, inside of throat dark purple, with pubescent annulate; 2-lipped, villous outside, upper lip oblong, erect and galeate, apex undulate or bilobate, ca. 8 mm long, ca. 4 mm wide; lower lip reflexed, ca. 4.5 mm long, 3-lobed, medium lobe largest, suborbicular, ca. 5 mm long, ca. 4.5 mm wide, apex emarginate, white, dotted with purplish-red

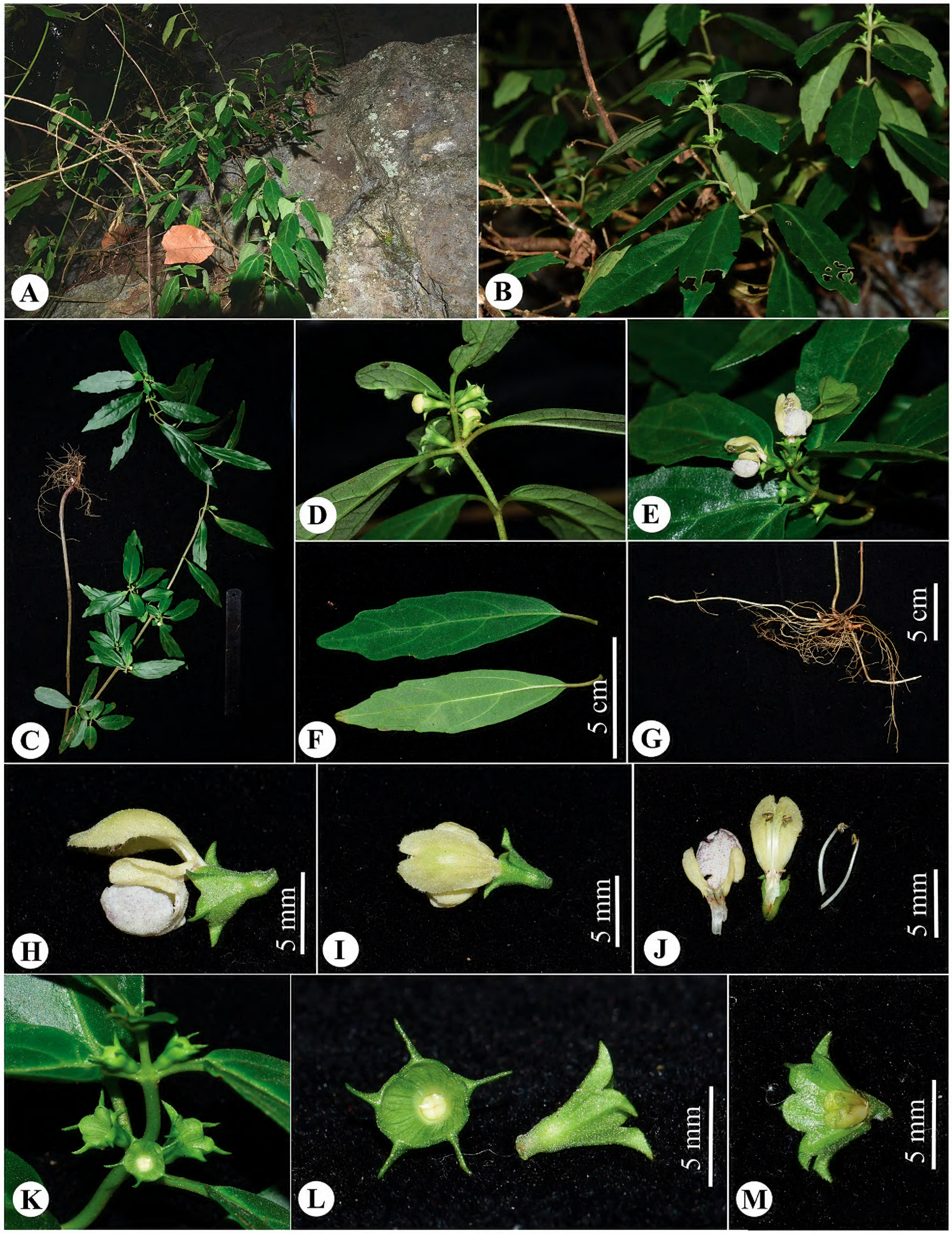

Figure 2. *Paraphlomis qingyuanensis* **A** habitat **B** plants **C** individual **D** inflorescences with unopened flowers **E** opening flowers **F** leaves **G** stolons and roots **H** lateral view of flower **I** upper lip of flower **J** dissected corolla and stamens **K** infructescence **L** frontal and lateral view of calyx **M** dissected calyx and seeds. (Photographs: **A, B** by Qin-Dai Xiong; **C–G** by Wan-Yi Zhao).

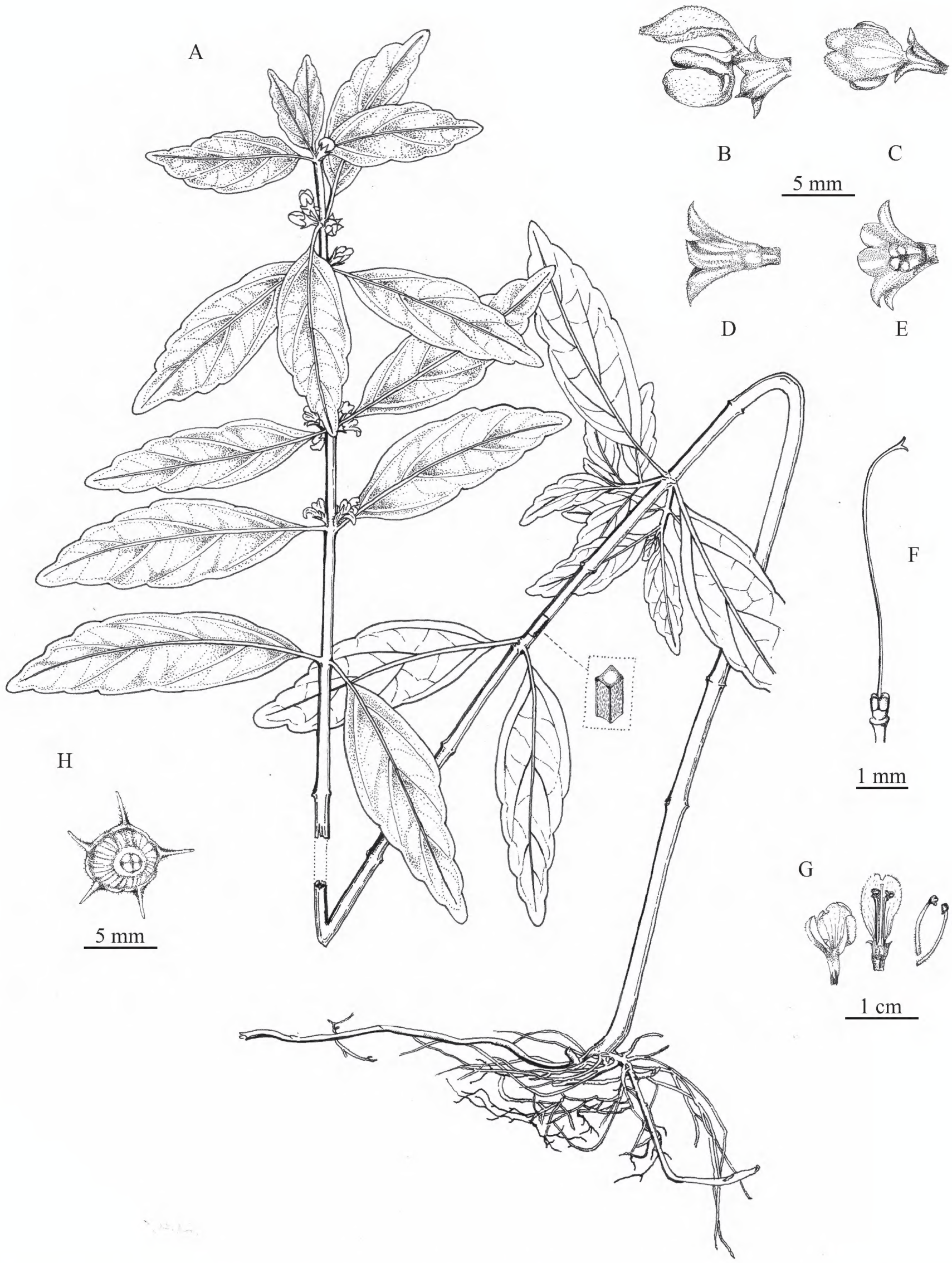

Figure 3. Line drawing of *Paraphlomis qingyuanensis* **A** plant **B** lateral view of flower **C** upper lip of flower **D** lateral view of calyx **E** inner view of calyx **F** style **G** dissected corolla and stamens **H** front view of calyx (Drawn by Zhong-Jin Wang).

spots, lateral lobes ovate, ca. 3.5 mm long, ca. 3 mm wide, apex obtuse, yellow. ***Stamens*** 4, inserted above middle and upper of corolla tube, straight, included, filaments flat, sparsely puberulent-villous, anther cells 2, ovoid, glabrous. ***Style*** filiform, included, glabrous, apex subequally 2-lobed, ca. 1 cm long.

Ovary 4-loculed, truncate at apex. ***Nutlets*** triquetrous-oblong, ca. 2.5 mm long, apex sparsely hispid and glandular.

Distribution, habitat and conservation status. Currently, only three populations of *Paraphlomis qingyuanensis* were found in Huanghua Town of Yingde City, Qingyuan City in Guangdong Province (Fig. 7A). These populations were located in the subtropical monsoon climate region, in a large area of karst landform. The distribution area of *P. qingyuanensis* is extremely fragmented, and it is not within a protected area. Human activity, such as forestry production and tourism, have a negative effect on population regeneration. Thus, *P. qingyuanensis* is here suggested to be endangered (EN) according to IUCN categories guidelines B2(a, b(iii)) (AOO < 500 km^2, number of locations <5, and habitat affected by human activities) (IUCN Standards and Petitions Subcommittee 2022).

Phenology. Flowering was observed from August to December, and fruiting from September to December.

Etymology. The specific epithet "*qingyuanensis*" is derived from the type locality of the new species, i.e. Qingyaun City in Guangdong Province, China.

Additional specimens examined. *Paraphlomis qingyuanensis* (paratypes): China. Guangdong Province: Qingyuan City, Yingde City, Huanghua Town, near Hegushi, on the limestone valley, 24°13'N, 112°56'E, alt. 132 m, 6 September 2023, *Qin-Dai Xiong QYK-HH-156* (SYS); Yingde city, Huanghua town, Hegushi, 24°13'35.7"N, 112°56'08.97"E, alt. 114 m, 16 December 2023, *Qiang Fan 20255* (SYS); Yingde city, Huanghua town, near Hegushi, 24°13'22.71"N, 112°56'05.71"E, alt. 159 m, 26 December 2023, *Qiang Fan & Qin-Dai Xiong QYK-HH-1904* (SYS); Yingde city, Huanghua town, Huanghua park, 24°12'03.46"N, 112°54'10.05"E, alt. 205 m, 25 December 2023, *Qiang Fan & Qin-Dai Xiong QYK-HH-1882* (SYS).

Paraphlomis baiwanensis **W.Y.Zhao, Y.P.Chen & Q.Fan, sp. nov.**
urn:lsid:ipni.org:names:77344363-1
Figs 4C, 5, 6
白湾假糙苏

Type. China. Guangdong Province: Qingyuan city, Qingxin district, Baiwan town, Hecang village, on the limestone cliff, 24°15'09.40"N, 112°46'47.32"E, alt. 310 m, 15 December 2023, *Qiang Fan 20251* (holotype: SYS00236952!; isotypes: KUN, SYS00236953!).

Diagnosis. *Paraphlomis baiwanensis* is morphologically similar to *P. subcoriacea*, but differs from the latter by its stout stem, larger and leathery leaves, and larger flower.

Description. ***Herbs*** perennial, 50–80 cm tall; stoloniferous, with villous indumentum. ***Stems*** erect, stout, unbranched or 2–3 branched, 4-angled, diameter 3.6–5.3 mm, with densely retrorse pilose hairs. ***Leaves*** opposite; lamina long ovate to lanceolate, leathery, 12.5–18.7 cm long, 2.6–4.8 cm wide, apex acuminate, base cuneate or abruptly obtuse, margin serrulate; adaxially dark green, with densely pilose, abaxially light green with densely brown glandular, sparsely pilose, more densely on veins; lateral veins 5–7 (-8)-paired, raised abaxially and deeply impressed adaxially, anastomosing at leaf margin; petiole 0.6–1.3 cm long, densely pilose. ***Verticillasters*** borne in upper leaf axils, cymes (2-) 5–9-flowered; bracteoles inconspicuous, early deciduous; pedicels ca. 1.2–2.0 mm long,

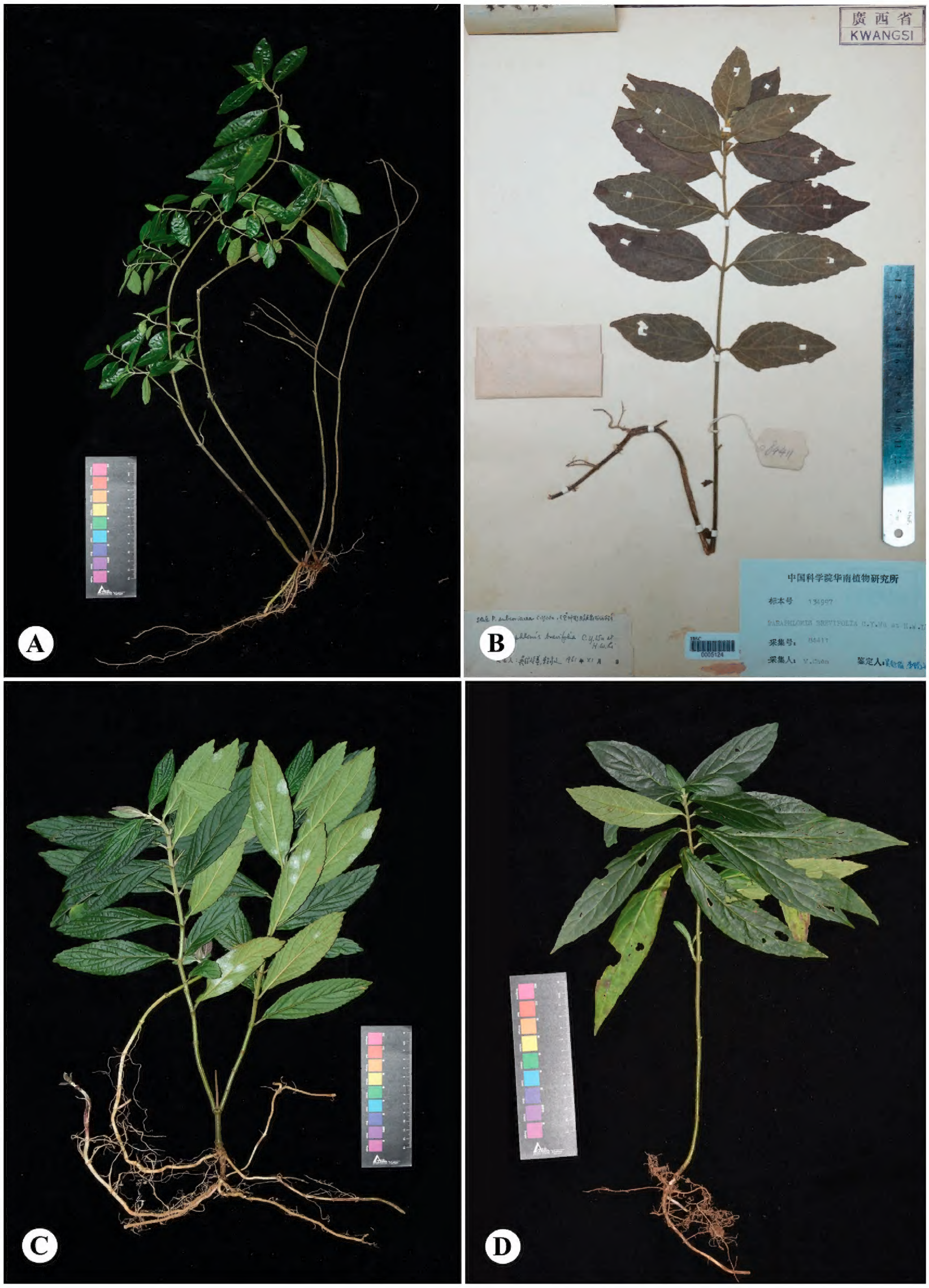

Figure 4. Morphological comparison of *Paraphlomis qingyuanensis*, *P. brevifolia*, *P. baiwanensis*, and *P. subcoriacea*. *Paraphlomis qingyuanensis* **A** plant of *P. qingyuanensis* **B** plant of *P. brevifolia* **C** plant of *P. baiwanensis* **D** plant of *P. subcoriacea* (Photographs by Wan-Yi Zhao; photographs of *P. brevifolia* from isotype *W. Chen 84411*, IBSC0005124).

densely pilose. ***Calyx*** light green, obconical, 5.2–5.7 mm long, glabrous inside, outside with densely retrorse pilose, conspicuously 10-veined; calyx teeth 5, inconspicuous, throat appearing subtruncate, calyx veins extended into wings as 2.5–3.0 mm long. ***Corolla*** 1.5–2.1 cm long, white; tube ca. 7 mm long, ca. 2 mm

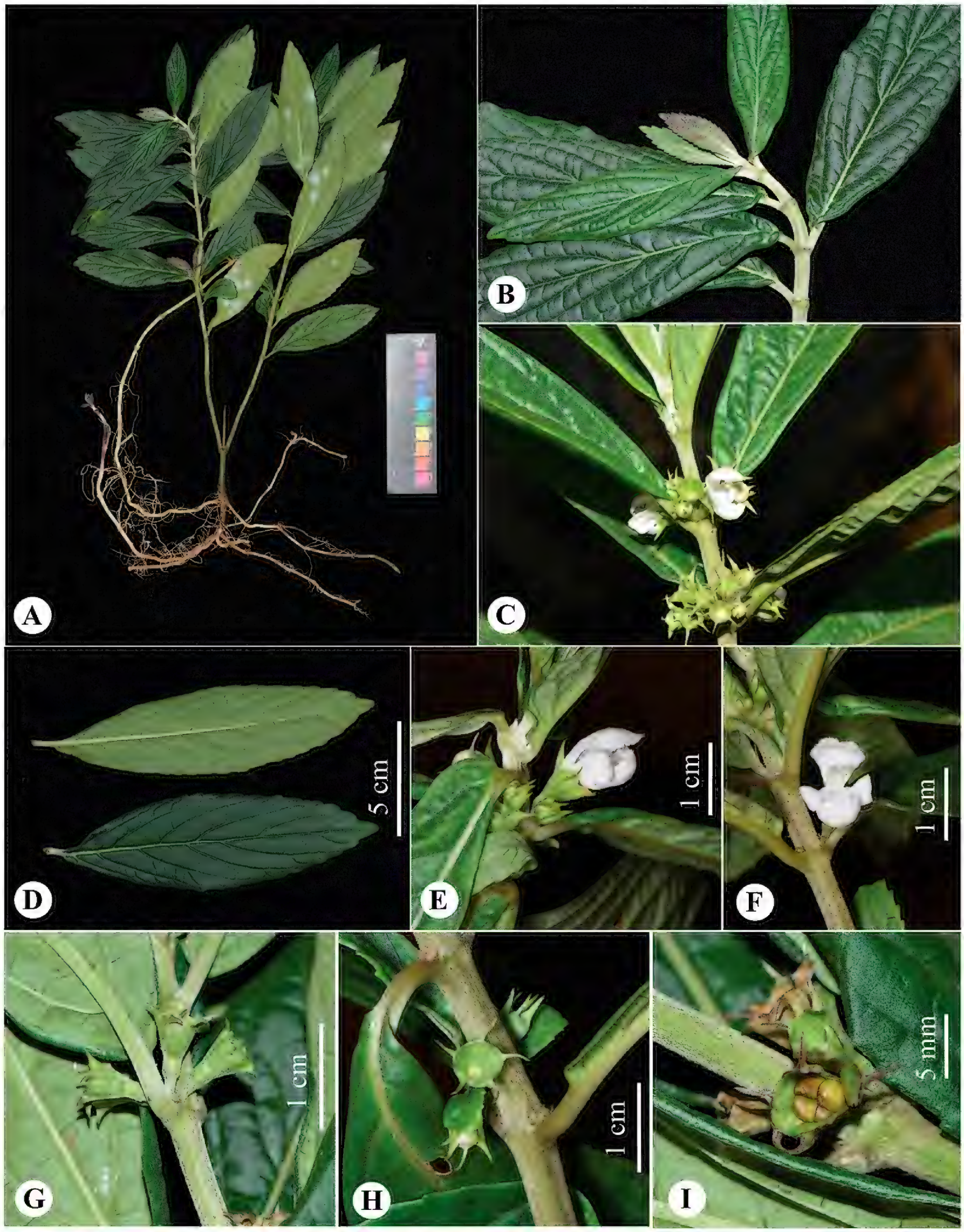

Figure 5. *Paraphlomis baiwanensis* **A** plant **B** young branch and leaves **C** flowering branch **D** leaves **E** lateral view of flower **F** front view of flower **G** lateral view of calyx **H** front view of calyx **I** seeds (Photographs: **A, B, D, G, I** by Wan-Yi Zhao; **C** by Qin-Dai Xiong; **E-F, H** by Qiang Fan).

in diam., straight, inside of throat with pubescent annulate; 2-lipped, villous outside, upper lip oblong, galeate, apex undulate, ca. 8–10 mm long, ca. 3.8–5.5 mm wide; lower lip reflexed, ca. 7 mm long, 3-lobed, medium lobe largest, suborbicular, ca. 6.5 mm long, ca. 6 mm wide, apex emarginate, lateral lobes ovate, ca.

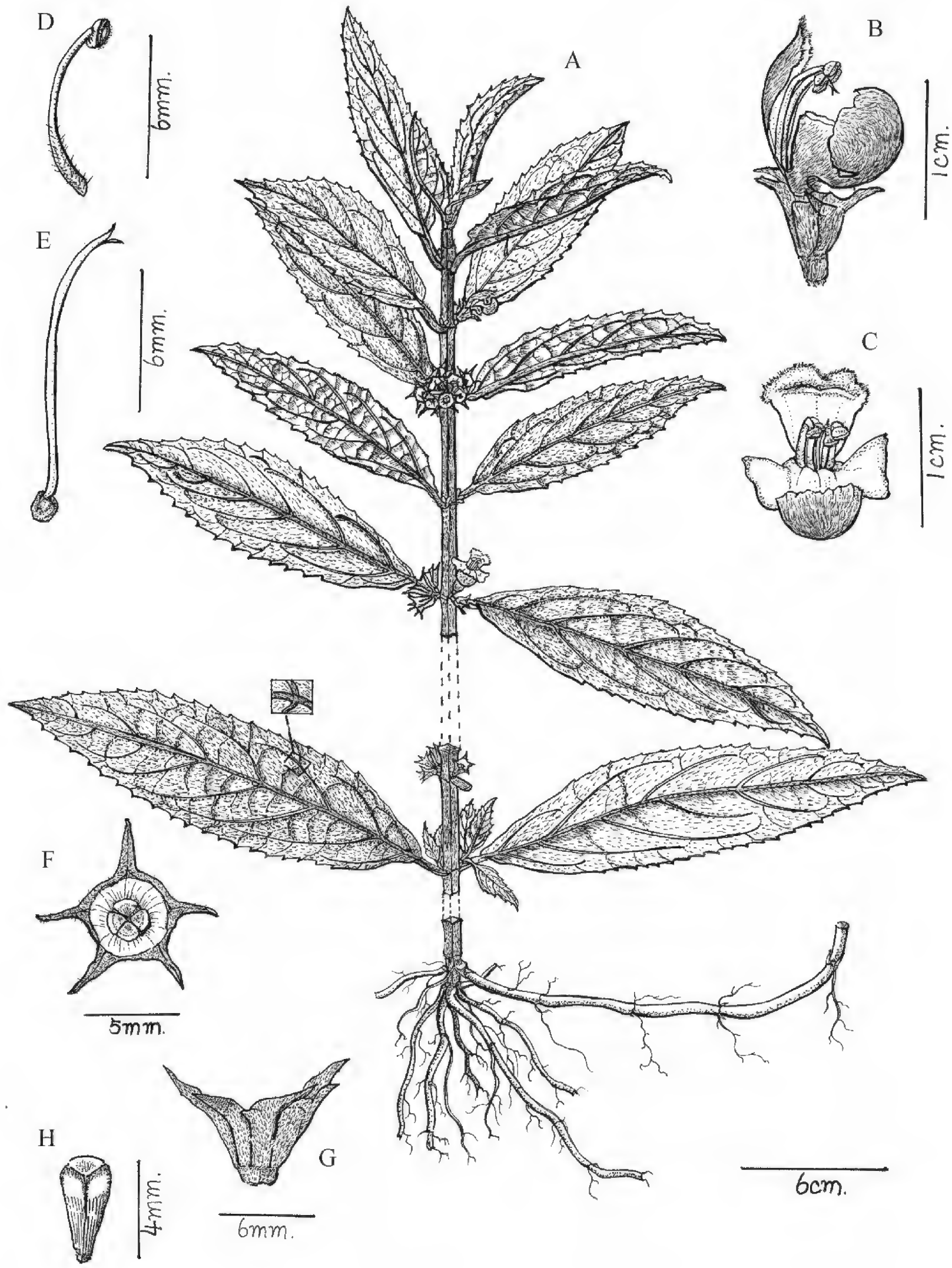

Figure 6. Line drawing of *Paraphlomis baiwanensis* **A** plant **B** lateral view of a flower **C** frontal view of corolla **D** stamen **E** style **F** front view of calyx **G** lateral view of calyx tube **H** seed (Drawn by Rong-En Wu).

4.5 mm long, ca. 3.2 mm wide, apex obtuse. ***Stamens*** 4, inserted above middle and upper of corolla tube, straight, included, filaments flat, 7–9 mm long, sparsely puberulent-villous, anther cells 2, ovoid, glabrous. ***Style*** filiform, included, glabrous, apex subequally 2-lobed, ca. 1 cm long. ***Ovary*** 4-loculed, truncate at apex. ***Nutlets*** triquetrous-oblong, ca. 3.8 mm long, apex sparsely hispid.

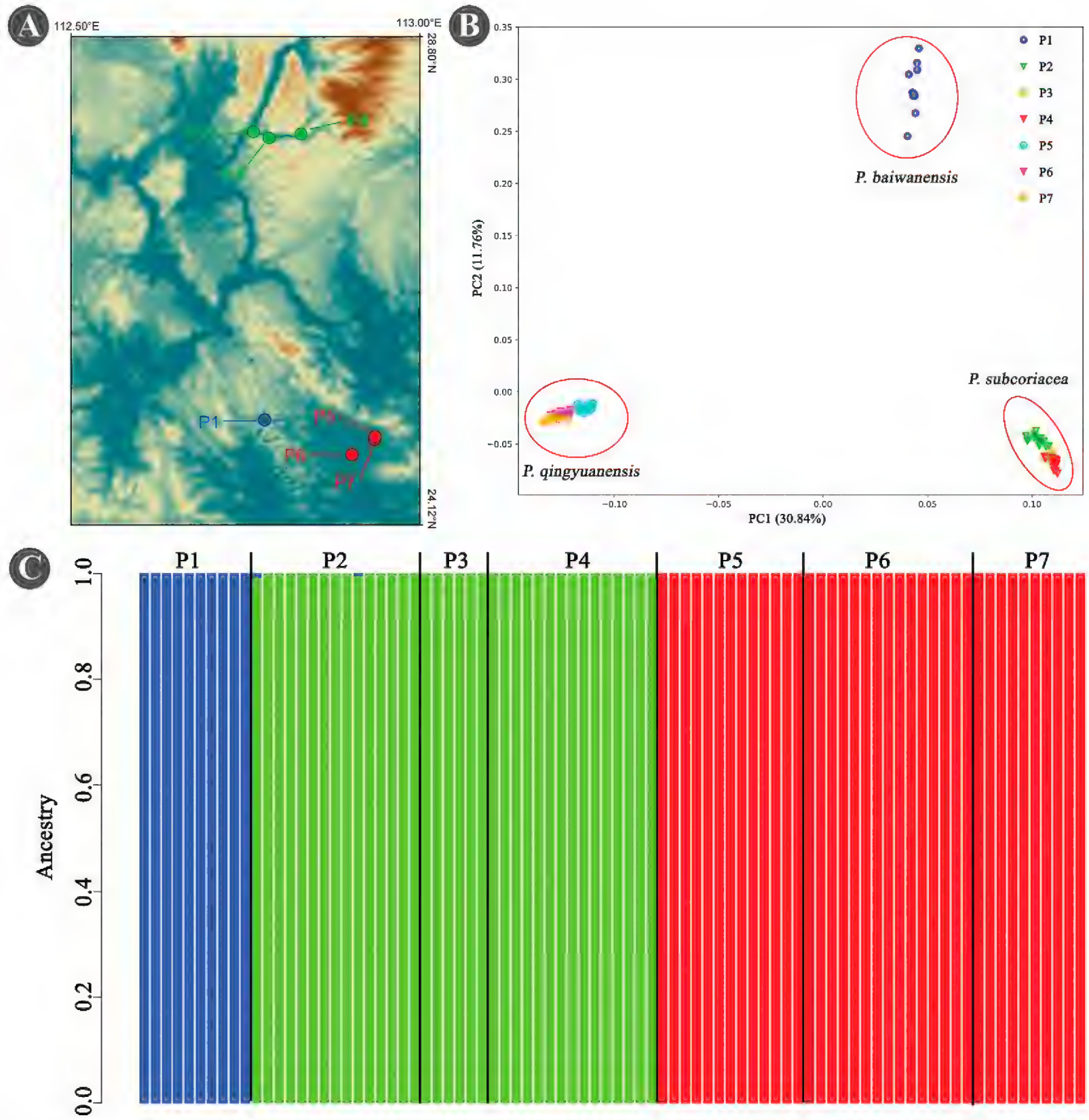

Figure 7. Geographic distribution and population genetic structure of *Paraphlomis baiwanensis* (P1), *P. subcoriacea* (P2–4), and *P. qingyuanensis* (P5–7) based on Admixture analysis **A** geographic distribution of the seven populations used for analysis **B** two-dimensional clustering of genetic variation from seven populations obtained via principal component analysis **C** Populations genetic structure result based on Admixture analysis.

Distribution, habitat and conservation status. *Paraphlomis baiwanensis* is currently known to occur only in Baiwan town, Guangdong in one population numbering less than one hundred individuals. It was observed to grow on limestone cliffs at altitudes about 300 m. Its known population was located in Qingxin Baiwan Provincial Nature Reserve of Guangdong which is well-protected. More field investigations are needed to determine its wild distribution. Therefore, the Protection level of *Paraphlomis baiwanensis* was suggested as Data Deficient (DD) based on the IUCN Red List Criteria (IUCN Standards and Petitions Subcommittee 2022).

Phenology. Flowering was observed from June to September, and fruiting from August to December.

Etymology. The specific epithet "*baiwanensis*" is derived from the type locality of the new species, i.e. Qingxin Baiwan Provincial Nature Reserve of Guangdong, Qingyuan, China.

Additional specimens examined. *Paraphlomis baiwanensis* (paratypes): China. Guangdong Province: Qingyuan city, Qingxin district, Baiwan town, Hecang village, on the limestone cliff, 24°15'09.40"N, 112°46'47.32"E, alt. 310 m, 23 August 2023,*Yi-Wen Liu QYK-BW-1470* (SYS).

Paraphlomis brevifolia: China. Guangxi Province: Luocheng City, 1939, *Wen Chen 84411* (IBSC0005124, isotype).

Paraphlomis subcoriacea: China. Guangdong Province: Qingyuan City, Yangshan City, Chengjia country, Wuyuan village, Baizhushan, 20 June 1956, *Liang Deng 1572* (IBSC0005130, isotype); Yangshan City, Dalang Town, Chakeng village, 7 August 1936, *Liang Deng 263* (IBK00059958; IBSC0585118); Yangshan City, Lingbei Town, Xiatianxia, 24°39'48.99"N, 112°45'41.73"E, alt. 295 m, 21 December 2023, *Qiang Fan 20257* (SYS); Yangshan City, Lingbei Town, Yanzidong, 24°39'16.92"N, 112°46'59.95"E, alt. 156 m, 27 December 2023, *Qiang Fan & Qin-Dai Xiong QYK-LB-1920* (SYS); Yangshan City, Chengjia Town, Baizhuling (collection site of type specimen), 24°39'39.56"N, 112°49'45.69"E, alt. 387 m, 27 December 2023, *Qiang Fan & Qin-Dai Xiong QYK-CJ-1911* (SYS).

Acknowledgements

We thank Fu-Gang Li, Fu-Zhen Liu from Yingde Forestry Bureau and Xin-Yu Jia from Guangdong Qingxin Baiwan Provincial Nature Reserve for their help in the field work. We are deeply grateful to Zhong-Jin Wang, Rong-En Wu for their excellent illustrations in the manuscript.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was supported by the Foundation of Qingyuan Forestry Bureau (HT-99982024-0030), Guangdong Provincial Ecological Forestry Development Project (2020141), the Yunnan Fundamental Research Project (202301AT070303), and Nanling Ecosystem Monitoring Project (GDEEMC-2023-19).

Author contributions

Q.F conceptualized and led the project. Field investigation and sample collection were conducted by Q.D.X, Q.F, R.M.W, Y.H.Z, and Z.B.X. W.YZ, and Q.D.X examined and detailed the fine structure of the species represented in the study. The phylogenetic analysis and population genetic analysis were carried out by Y.P.C, and Q.F. The initial draft of the manuscript was prepared by W.YZ and Y.P.C. All the authors approved the final manuscript.

Author ORCIDs

Wan-Yi Zhao https://orcid.org/0000-0002-6103-1236
Qin-Dai Xiong https://orcid.org/0009-0006-3645-7362
Rang-Min Wu https://orcid.org/0009-0004-3545-1933

You-Hong Zeng https://orcid.org/0009-0004-1171-5762
Zhi-Bin Xie https://orcid.org/0009-0004-9382-8073
Ya-Ping Chen https://orcid.org/0000-0002-7502-1848
Qiang Fan https://orcid.org/0000-0003-4254-6936

Data availability

The newly-obtained ITS and ETS sequences have been submitted to the NCBI (accession number: PP897029–PP897034; PP897950–PP897955). RAD raw data was deposited to the NCBI SRA database with BioProject PRJNA1107389. All of the other data that support the findings of this study are available in the main text.

References

Alexander DH, Novembre J, Lange K (2009) Fast model-based estimation of ancestry in unrelated individuals. Genome Research 19(9): 1655–1664. https://doi.org/10.1101/gr.094052.109

Beardsley PM, Olmstead RG (2002) Redefining Phrymaceae: The placement of *Mimulus*, tribe Mimuleae, and *Phryma*. American Journal of Botany 89(7): 1093–1102. https://doi.org/10.3732/ajb.89.7.1093

Bendiksby M, Thorbek L, Scheen AC, Lindqvist C, Ryding O (2011) An updated phylogeny and classification of Lamiaceae subfamily Lamioideae. Taxon 60(2): 471–484. https://doi.org/10.1002/tax.602015

Brown JW, Walker JF, Smith SA (2017) Phyx: Phylogenetic tools for unix. Bioinformatics 33(12): 1886–1888. https://doi.org/10.1093/bioinformatics/btx063

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: An analysis tool set for population genomics. Molecular Ecology 22(11): 3124–3140. https://doi.org/10.1111/mec.12354

Chang CC, Chow CC, Tellier LCAM, Vattikuti S, Purcell SM, Lee JJ (2015) Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience 4(1): s13742-015-0047-8. https://doi.org/10.1186/s13742-015-0047-8

Chen YP, Drew BT, Li B, Soltis DE, Soltis PS, Xiang CL (2016) Resolving the phylogenetic position of *Ombrocharis* (Lamiaceae), with reference to the molecular phylogeny of tribe Elsholtzieae. Taxon 65(1): 123–136. https://doi.org/10.12705/651.8

Chen YP, Liu A, Yu XL, Xiang CL (2021) A preliminary phylogenetic study of *Paraphlomis* (Lamiaceae) based on molecular and morphological evidence. Plant Diversity 43(3): 206–215. https://doi.org/10.1016/j.pld.2021.03.002

Chen YP, Sun ZP, Xiao JF, Yan KJ, Xiang CL (2022a) *Paraphlomis longicalyx* (Lamiaceae), a new species from the limestone area of Guangxi and Guizhou Provinces, southern China. Systematic Botany 47(1): 251–258. https://doi.org/10.1600/036364422X16442668423572

Chen YP, Xiao JF, Xiang CL, Li X (2022b) *Paraphlomis hsiwenii* (Lamiaceae), a new species from the limestone area of Guangxi, China. PhytoKeys 212: 85–96. https://doi.org/10.3897/phytokeys.212.91174

Chen YP, Xiong C, Zhou HL, Chen F, Xiang CL (2022c) *Paraphlomis nana* (Lamiaceae), a new species from Chongqing, China. Turkish Journal of Botany 46(2): 176–182. https://doi.org/10.55730/1300-008X.2680

Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, McVean G, Durbin R (2011) The variant call format and VCFtools. Bioinformatics 27(15): 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Ding BY, Chen ZH, Xu YL, Jin XF, Wu DF, Chen JB, Wu WJ (2019) New species and combination of Lamiaceae from Zhejiang, China. Guihaia 39(1): 10–15.

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Guo GX, Zhao WY, Chen YP, Xiao JH, Li YQ, Fan Q (2023) *Paraphlomis yingdeensis* (Lamiaceae), a new species from Guangdong (China). PhytoKeys 219: 107–120. https://doi.org/10.3897/phytokeys.219.97547

IUCN Standards and Petitions Subcommittee (2022) Guidelines for Using the IUCN Red List Categories and Criteria. Version 15.1. Prepared by the Standards and Petitions Subcommittee. https://www.iucnredlist.org/resources/redlistguidelines

Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Ko SC, Lee YM, Chung KS, Son DC, Nam BM, Chung GY (2014) A new species of *Paraphlomis* (Lamiaceae) from Korea: An additional genus to the Korean flora. Phytotaxa 175(1): 51–54. https://doi.org/10.11646/phytotaxa.175.1.6

Li HW (1965) Revisio generis *Paraphlomis* Labiatarum sinensium. Acta Phytotaxonomica Sinica 10(1): 57–74.

Li HW, Hedge IC (1994) *Paraphlomis*. In: Wu CY, Raven PH (Eds) Flora of China, Vol. 17. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 170–177. https://www.iplant.cn/foc/pdf/Paraphlomis.pdf

Li B, Cantino PD, Olmstead RG, Bramley GLC, Xiang CL, Ma ZH, Tan YH, Zhang DX (2016) A large-scale chloroplast phylogeny of the Lamiaceae sheds new light on its subfamilial classification. Scientific Reports 6(1): 34343. https://doi.org/10.1038/srep34343

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA, 1–8. https://doi.org/10.1109/GCE.2010.5676129

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stover B, Müller K (2010) TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. BMC Bioinformatics 11(1): 1–9. https://doi.org/10.1186/1471-2105-11-7

Sun Y, Skinner DZ, Liang GH, Hulbert SH (1994) Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. Theoretical and Applied Genetics 89(1): 26–32. https://doi.org/10.1007/BF00226978

Thiers BM (2023) [continuously updated] Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/science/ih/ [Accessed 06.10.2023]

Wu CY, Li HW (1977) *Paraphlomis* Prain. In: Wu CY, Li HW (Eds) Flora Reipublicae Popularis Sinicae, vol. 65(2). Science Press, Beijing, 545–572.

Yan KJ, Fang D (2009) A supplement to the *Paraphlomis* (Lamiaceae) from Guangxi, China. Journal of Tropical and Subtropical Botany 17(7): 91–92. http://jtsb.ijournals.cn/jtsb_cn/article/issue/2009_17_1

Yan KJ, Huang YF, Li X, Hu RC, Wu WH, Chen YP (2023) *Paraphlomis caloneura* (Lamiaceae), A New Species From Guangxi, China. Systematic Botany 48(2): 346–353(8). https://doi.org/10.1600/036364423X16847773873198

Yuan JC, Chen YP, Zhao Y, Li HB, Chen B, Xiang CL (2022) *Paraphlomis strictiflora* (Lamioideae, Lamiaceae), a new species from Guizhou, China. Phytotaxa 575(3): 276–286. https://doi.org/10.11646/phytotaxa.575.3.5

Zhang RB, Deng T, Dou QL, Wei RX, He L, Ma CB, Zhao S, Hu S (2020) *Paraphlomis kuankuoshuiensis* (Lamiaceae), a new species from the limestone areas of northern Guizhou, China. PhytoKeys 139: 13–20. https://doi.org/10.3897/phytokeys.139.47055

Zhao F, Chen YP, Salmaki Y, Drew BT, Wilson TC, Scheen AC, Celep F, Bräuchler C, Bendiksby M, Wang Q, Min DZ, Peng H, Olmstead RG, Li B, Xiang CL (2021) An updated tribal classification of Lamiaceae based on plastome phylogenomics. BMC Biology 19(1): 2. https://doi.org/10.1186/s12915-020-00931-z

Zhao WY, Zhang Z, Fan Q, Chen CQ, Liao WB, Boufford DE (2022) *Paraphlomis jinggangshanensis* (Lamiaceae), a new species from Jiangxi, China. PhytoKeys 204: 1–8. https://doi.org/10.3897/phytokeys.204.87654

Appendix 1

Table A1. Sequence information for all samples used in present study. A "/" indicates a missing sequence. Herbarium abbreviations are listed after the vouchers. The accession numbers marked in bold represent sequences newly generated.

Taxon	Voucher	Country	ITS	ETS
Matsumurella chinensis (Benth.) Bendiksby 1	Y. Yang OYY00316 (KUN)	Pingxiang, Jiangxi, China	MW602147	MW602117
Matsumurella chinensis (Benth.) Bendiksby 2	Y. Yang OYY00131 (KUN)	Guilin, Guangxi, China	MW602148	MW602118
Matsumurella yangsoensis (Y.Z. Sun) Bendiksby	L. Wu & W.B. Xu 10965 (IBK)	Yangshuo, Guangxi, China	MW602142	MW602112
Paraphlomis albida Hand.-Mazz. var. *albida*	A. Liu et al. LK0841 (CSFI)	Ningyuan, Hunan, China	MW602124	MW602091
Paraphlomis albida var. *brevidens* Hand.-Mazz.	Y.P. Chen EM312 (KUN)	Hezhou, Guangxi, China	MW602130	MW602098
Paraphlomis albiflora (Hemsl.) Hand.-Mazz.	C.M. Tan et al. 1806393 (JJF)	Jiujiang, Jiangxi, China	/	MW602101
***Paraphlomis baiwanensis* 1**	**Y.S. Chen et al. QY20230302 (IBSC)**	**Qingyuan, Guangdong, China**	**PP897029**	**PP897950**
***Paraphlomis baiwanensis* 2**	**Q. Fan et al. 20251 (SYS)**	**Qingyuan, Guangdong, China**	**PP897030**	**PP897951**
Paraphlomis caloneura K.J. Yan, Y.P. Chen & Y.F. Huang	W.H. Wu et al. LHT1841 (KUN)	Napo, Guangxi, China	OQ627454	OQ628080
Paraphlomis coronata (Vaniot) Y.P. Chen & C.L. Xiang 1	E.D. Liu et al. 3043 (KUN)	Emeishan, Sichuan, China	MW602137	MW602107
Paraphlomis coronata (Vaniot) Y.P. Chen & C.L. Xiang 2	C.L. Xiang 358 (KUN)	Jiangkou, Guizhou, China	MW602123	MW602090
Paraphlomis foliata (Dunn) C.Y. Wu & H.W. Li subsp. *foliata*	S.P. Chen s.n. (KUN)	Jiangle, Fujian, China	/	MW602097
Paraphlomis foliata subsp. *montigena* X.H. Guo & S.B. Zhou	Y.C. Dai s.n. (KUN)	Hangzhou, Zhejiang, China	OM836064	OM884453
Paraphlomis gracilis (Hemsl.) Kudô var. gracilis 1	A. Liu LK0931 (CSFI)	Changsha, Hunan, China	MW602134	MW602104
Paraphlomis gracilis (Hemsl.) Kudô var. *gracilis 2*	C.L. Xiang XCL1315 (KUN)	Chongqing, China	MW602141	MW602111
Paraphlomis gracilis var. *lutienensis* (Y.Z. Sun) C.Y. Wu	C.L. Xiang XCL881 (KUN)	Shibing, Guizhou, China	MW602131	MW602099
Paraphlomis hirsutissima C.Y. Wu & H.W. Li	F. Zhao & G. Chen XCL2115 (KUN)	Malipo, Yunnan, China	OQ627453	OQ628079
Paraphlomis hispida C.Y. Wu	X. Li LX200702 (GXF)	Napo, Guangxi, China	MW602132	MW602102
Paraphlomis hsiwenii Y.P. Chen & Xiong Li 1	W.H. Wu et al. DD426 (KUN)	Jingxi, Guangxi, China	OP605346	OP609841
Paraphlomis hsiwenii Y.P. Chen & Xiong Li 2	W.H. Wu et al. DD426 (KUN)	Jingxi, Guangxi, China	OP605347	OP609842
Paraphlomis intermedia C.Y. Wu & H.W. Li	X. Zhong et al. ZX16823 (CSH)	Suichang, Zhejiang, China	MW602135	MW602105
Paraphlomis javanica (Blume) Prain var. *javanica* 1	Y.P. Chen s.n. (KUN)	Kunming, Yunnan, China	MW602121	MW602088
Paraphlomis javanica (Blume) Prain var. *javanica* 2	L.B. Jia et al. JLB0029 (KUN)	Maguan, Yunnan, China	MW602143	MW602113
Paraphlomis javanica var. *pteropoda* D. Fang & K.J. Yan	X. Li 2020090501 (GXF)	Jingxi, Guangxi, China	MW602140	MW602110
Paraphlomis jiangyongensis X.L. Yu & A. Liu 1	A. Liu et al. LK1104 (CSFI)	Jiangyong, Hunan, China	MW602128	MW602095

Taxon	Voucher	Country	ITS	ETS
Paraphlomis jiangyongensis X.L. Yu & A. Liu 2	A. Liu et al. LK1104 (CSFI)	Jiangyong, Hunan, China	MW602129	MW602096
Paraphlomis kwangtungensis C.Y. Wu & H.W. Li	Q. Fan et al. 19738 (SYS)	Qujiang, Guangdong, China	PP713070	PP706067
Paraphlomis lanceolata Hand.-Mazz. 1	C.Z. Huang s.n. (KUN)	Guidong, Hunan, China	MW602145	MW602115
Paraphlomis lanceolata Hand.-Mazz. 2	A. Liu et al. LK0825 (CSFI)	Ningyuan, Hunan, China	MW602146	MW602116
Paraphlomis lancidentata Y.Z. Sun	X. Zhong et al. ZX16824 (CSH)	Suichang, Zhejiang, China	MW602136	MW602106
Paraphlomis longicalyx Y.P. Chen & C.L. Xiang	Y.P. Chen et al. EM583 (KUN)	Huanjiang, Guangxi, China	OK104771	OK104774
Paraphlomis membranacea C.Y. Wu & H.W. Li	M.S. Nuraliev 1057 (MW)	Thanh Son, Phu Tho, Vietnam	/	MW602100
Paraphlomis nana Y.P. Chen, C. Xiong & C.L. Xiang 1	C. Xiong XC21097 (KUN)	Chengkou, Chongqing, China	OM836062	OM884451
Paraphlomis nana Y.P. Chen, C. Xiong & C.L. Xiang 2	C. Xiong & H.L. Zhou XC21126 (KUN)	Wushan, Chongqing, China	OM836063	OM884452
Paraphlomis octopus Q. Fan, Y.P. Chen & Ying Liu 1	Y.P. Chen & Y. Zhao EM1391 (KUN)	Huaiji, Guangdong, China	MW602126	MW602093
Paraphlomis octopus Q. Fan, Y.P. Chen & Ying Liu 2	Q. Fan et al. 19752 (SYS)	Fengkai, Guangdong, China	PP713071	PP706068
Paraphlomis octopus Q. Fan, Y.P. Chen & Ying Liu 3	Q. Fan et al. 19760 (SYS)	Pingle, Guangxi, China	PP713072	PP706069
Paraphlomis pagantha Dunn 1	L.X. Yuan et al. s.n. (KUN)	Qionghai, Hainan, China	OP605345	OP609840
Paraphlomis pagantha Dunn 2	X.Y. Jiang et al. HN001 (SYS)	Wenchang, Hainan, China	PP713073	PP706070
Paraphlomis patentisetulosa C.Y. Wu	C.L. Su et al. XY015 (KUN)	Xinyi, Guangdong, China	OQ627455	OQ628081
Paraphlomis paucisetosa C.Y. Wu 1	X.X. Zhu s.n. (KUN)	Malipo, Yunnan, China	MW602125	MW602092
Paraphlomis paucisetosa C.Y. Wu 2	X. Li LX200704 (GXF)	Napo, Guangxi, China	MW602133	MW602103
***Paraphlomis qingyuanensis* 1**	**Q. Fan et al. QYK-HH-1904 (SYS)**	**Yingde, Guangdong, China**	**PP897031**	**PP897952**
***Paraphlomis qingyuanensis* 2**	**Q. Fan et al. 20255 (SYS)**	**Yingde, Guangdong, China**	**PP897032**	**PP897953**
Paraphlomis reflexa C.Y. Wu & H.W. Li	Z.Z. Yang et al. s.n. (HIB)	Tongshan, Hubei, China	MW602122	MW602089
Paraphlomis strictiflora J.C. Yuan, B. Chen & C.L. Xiang	B. Chen et al. CB05956 (CSH)	Yinjiang, Guizhou, China	/	OP609839
***Paraphlomis subcoriacea* 1**	**Q. Fan et al. QYK-CJ-1911 (SYS)**	**Yangshan, Guangdong, China**	**PP897033**	**PP897954**
***Paraphlomis subcoriacea* 2**	**Q. Fan et al. QYK-LB-1920 (SYS)**	**Yangshan, Guangdong, China**	**PP897034**	**PP897955**
Paraphlomis yingdeensis W.Y. Zhao, Y.Q. Li & Q. Fan	Q. Fan et al. 19013 (SYS)	Yingde, Guangdong, China	OP605348	OP609843
Phlomis fruticosa L.	Y. Tong s.n. (KUN)	Shanghai, China (cultivated)	MW602119	MW602086
Phlomoides dentosa var. *glabrescens* (Danguy) C.L. Xiang & H. Peng	Y.P. Chen EM360 (KUN)	Beijing, China (cultivated)	MW602120	MW602087

Appendix 2

Table A2. Populations information for PCA and Admixture analysis used in this study.

Population ID	Species	Voucher	GPS	Number of individual
P1	*Paraphlomis baiwanensis*	Q. Fan 20251 (SYS); Hecang village, Baiwan town, Qingyuan city, Guangdong, China	24°15'09.40"N, 112°46'47.32"E	10
P2	*Paraphlomis subcoriacea*	Q. Fan 20257 (SYS); Xiatianxia, Lingbei town, Yangshan city, Guangdong, China	24°39'48.99"N, 112°45'41.73"E	15
P3	*Paraphlomis subcoriacea*	QYK-LB-1920 (SYS); Yanzidong, Lingbei town, Yangshan city, Guangdong, China	24°39'16.92"N, 112°46'59.95"E	6
P4	*Paraphlomis subcoriacea*	QYK-CJ-1911 (SYS); Baizhuling, Chengjia country, Yangshan city, Guangdong, China	24°39'39.56"N, 112°49'45.69"E	15
P5	*Paraphlomis qingyuanensis*	Q. Fan 20255 (SYS); Hegushi, Huanghua town, Yingde city, Guangdong, China	24°13'35.7"N, 112°56'08.97"E	14
P6	*Paraphlomis qingyuanensis*	QYK-HH-1882 (SYS); Huanghua park, Huanghua town, Yingde city, Guangdong, China	24°12'03.46"N, 112°54'10.05"E	15
P7	*Paraphlomis qingyuanensis*	QYK-HH-1904 (SYS); near Hegushi, Huanghua town, Yingde city, Guangdong, China	24°13'22.71"N, 112°56'05.71"E	10